CDC Protocol for Measles Virus Isolation

(Adapted from Appendix 6: Measles Virus Isolation from the *CDC's Manual for the Surveillance of Vaccine-Preventable Diseases*)

Background

The availability of a sensitive cell line (B95a) for isolation of measles virus from clinical specimens and the establishment of automated DNA sequencing techniques have allowed for rapid genetic characterization of a large number of wild-type strains of measles virus. This database of sequence information now makes it possible to use molecular epidemiological techniques to identify the source of wild-type viruses and to rapidly differentiate between wild-type and vaccine strains. As progress is made toward elimination of measles in the U.S., it will be critical to examine virus isolates from as many outbreaks and isolated cases as possible in order to identify the source of the virus.

Virus isolation and genetic characterization can take several weeks to complete. Therefore, laboratory diagnosis of measles should always be based on detection of measles-specific IgM in serum. The IgM-capture EIA test can be completed in one day, and is available from virtually all state health department laboratories and commercial sources. Specimens for virus isolation should be taken at the same time that serum is obtained, since a delay in collection will reduce the chance of isolating the virus. However, urine or respiratory specimens should not be substituted for serum specimens for measles diagnosis.

The Measles Virus Section, CDC is currently testing the possibility of using saliva in addition to serum to diagnose measles using the IgM-capture EIA. During a measles outbreak, if you believe that you can collect saliva in addition to serum to help test this method, please contact the Measles Virus Section (contact information provided below), who will provide you with saliva collection kits. An aliquot of serum should be sent with the saliva.
Direct PCR from clinical specimens has improved the ability to provide genotyping information in many instances when virus culture was unsuccessful. However, isolation of virus is always preferable since it is the most reliable, direct evidence for measles virus infection. Isolated viruses can be used for virological analyses and further genetic characterization. **Therefore, isolation of virus is always attempted first.**

Although viral RNA in the clinical sample may survive short periods of time at ambient temperature, the RNA is subject to degradation. The best results using direct PCR are from samples that have been collected within 1-5 days of rash and have been kept cold or frozen at -70° C.

The possibility for direct PCR detection and sequencing from specimens which do not produce virus in culture means that this technique can play an important role in surveillance of measles virus. This is especially true when a sporadic suspected measles case occurs and no other specimens are available. For this reason, laboratories which perform measles virus isolation should save an aliquot of the original clinical material that can be tested later by PCR at CDC or in a laboratory that has this capability.

Protocols for isolation of measles virus

Specimens for virus isolation should be obtained as soon as possible after the onset of rash. Always collect a urine specimen and, if possible, attempt to collect a respiratory specimen.

Respiratory Specimens

Materials:

- sterile swabs
- sterile saline
- 3 ml aliquots of viral transport medium (VTM)
    - VTM: sterile PBS or suitable isotonic solution such as Hank's BSS, etc. containing antibiotics (100 units/ml penicillin, 100 µg/ml streptomycin) and either 2 % fetal bovine serum or 0.5% gelatin in 15 ml polycarbonate or polystyrene centrifuge tubes
- 5 ml plastic syringes
- plastic aspirators or 30 ml syringe
- Styrofoam shipping containers

Instructions:

1. Attempt to obtain the sample as soon as possible after onset of rash. Samples collected 5 days after rash onset have much lower chances for successful isolation of virus.
2. The preferred respiratory specimen is a nasal wash (nasopharyngeal aspirate) using a syringe attached to a small piece of plastic tubing and about 3 ml of sterile saline. After placing saline in the nose, aspirate as much of the material as possible and add to the centrifuge tube containing the VTM. Rinse the syringe and collection tubing into the VTM.
3. Alternatively, sterile swabs can be used to wipe the nose and throat. Place both swabs in a tube containing 2-3 ml of VTM. The virus is extremely cell-associated, so attempt to swab the throat and nasal passages to collect epithelial cells. If the specimen is to be shipped without freezing, the swab can be left in the tube of VTM.
4. Keep all specimens on wet ice or at 4° C and ship as soon as possible on cold packs (see address below).

Note: If immediate, cold shipment (within 48 hrs) cannot be arranged or is not convenient, nose and throat swabs should be removed from the VTM. Gently vortex or swirl the swab in the fluid and ream the swab against the side of the tube. These samples should be frozen and shipped at -70° C (dry ice).

Urine specimens

Instructions:

1. The best results for virus recovery are from urine samples collected within 5 days of rash onset, though urine collected up to 16 days after rash onset will be accepted. Virus has been isolated from the urine for up to one week after the onset of rash. In addition, CDC will accept urine collected from close contacts of measles cases (e.g., household contacts).
2. First morning voided specimens are ideal, but any urine collection is adequate. Collect 10-50 ml of urine in a urine specimen container.
3. It is best to centrifuge the urine specimen as soon after collection as possible. After collection, keep the specimen cool (refrigerator or wet ice). If facilities are available, centrifuge the urine at 400 x g for 10 minutes at 4° C to pellet the sediment. Resuspend the sediment in 2 ml of VTM (above) or any cell culture medium (DMEM, EMEM, RPMI plus antibiotics) and ship. Preferably, specimens that have been centrifuged and resuspended should be frozen at -70° C and shipped on dry ice. If dry ice is not available, however, they

can be stored at 4° C and shipped on cold packs.

4. Avoid repeated freeze-thaw cycles. If centrifugation is not available, do not freeze the urine sample. The entire urine specimen should be stored at 4° C, and shipped to the lab on cold packs. Most urine collection cups are not leak-proof. Transfer the urine to sterile plastic centrifuge tubes.

Shipping

All samples to CDC should be sent through the Utah Public Health Laboratory (UPHL). UPHL will ship specimens to CDC after consultation with the Utah Department of Health Bureau of Epidemiology.

Specimens should be sent to:

Utah Public Health Laboratory
46 North Medical Drive
Salt Lake City, UT 84113

Reporting

Specimen submission to CDC for virus isolation should be coordinated through the Utah Department of Health Bureau of Epidemiology, which can be contacted at (801) 538-6191 (24/7).